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Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	4/18/94	FINAL 2/91-3/94

4. TITLE AND SUBTITLE

SPIDER SILK PROTEINS

5. FUNDING NUMBERS

DAAL03-91-G-0044

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9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U. S. Army Research Office
P. O. Box 12211
Research Triangle Park, NC 27709-2211

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

ARO 28457.8-LS

11. SUPPLEMENTARY NOTES

The view, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.

12a. DISTRIBUTION/AVAILABILITY STATEMENT

Approved for public release; distribution unlimited.

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

Spider silk proteins from major and minor ampullate silk have been sequenced via their cDNAs. One of the proteins from major ampullate silk has been expressed in bacteria to levels of 10-20 mg/mL. Studies using fiber X-ray diffraction and solid state NMR have been used to study the structure of the proteins in the fiber.

14. SUBJECT TERMS	15. NUMBER OF PAGES
spider, silk, proteins, expression, X-ray diffraction NMR	3
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UL

A. Spider silk fibers possess a number of unique properties when compared to other natural or manmade fibers. These include: a) tensile strength (above 200,000 psi), greater than steel; b) elasticity higher than high elastic nylon(up to 200%); c) a relatively low stiffness modulus(similar to silkworm silk); and d) supercontraction(up to 50% dry length) in water but not organic solvents. The typical orb-web spider produces at least six different types of silk each of which is produced in a separate gland. The silk fibers appear to be composed entirely of protein, as no other major component has been identified. Our goal is to study these proteins to determine what gives them the properties observed and what the relationships are between the protein structure and the functional properties.

B. We have cloned and sequenced cDNAs encoding two proteins comprising major ampullate silk. Based on the sequences of those proteins and various biophysical measurements we have proposed a model we call the Lego-Slinky model to explain the structure/function relationship. The model proposes that regions of the protein contain molecular analogs to the Lego blocks which can insert into other protein molecules and hold them together. This results in the tensile strength. Other segments of the protein form coils like the Slinky toy which can expand and contract when force is applied resulting in elasticity.

We have constructed synthetic genes for the consensus sequence (4,8,16, and 32 repeats) of the second protein, Spidroin 2, which have been shown to produce the correct protein in an in vitro translation system. Currently we are working on overproducing the proteins in E.coli. and constructing synthetic genes for Spidroin 1 consensus repeats. The protein for the 16 repeat has been produced in milligram quantities from 500mL cultures and the protein has the correct molecular weight and amino acid composition. Further studies of the protein are currently planned.

We have tested major and minor ampullate and cocoon silks of both Nephila clavipes and Araneus gemmoides using standardized mechanical testing methods. We found the silks to exceed the published data for tensile strength by a substantial margin when the average diameter used is the minimum diameter at ten points not the average from the density, length and weight calculation. This minimum diameter is about 60% of the average diameter and since it is most likely the silks break at the narrowest point, these values may be more characteristic of the properties of the silk fibers.

We have cloned and sequenced the cDNA for a protein from minor ampullate silk. The sequences obtained show some similarity to both major ampullate silk proteins but have substantial differences. We also have the 5' or amino terminal end as well as the 3' end through the untranslated region. We have found what appears to be a second minor ampullate silk protein just in the past couple of weeks. Preliminary sequencing indicates it is clearly distinct from the first protein but similar in many respects. The complete 3' and 5' ends of this clone appear to be present.

The cocoon silk has proven to be more difficult to clone but we feel we have clones for it although they are relatively short. During the cloning of the minor ampullate silk we devised a strategy which may prove successful for this silk clone as well. We use

biased random hexamers as well as the usual poly dT for priming and it appears to yield a much greater number of silk clones as well as longer ones. This is currently being attempted on the cocoon gland mRNA.

We have started doing solid state NMR on both synthetic peptides based on repeats of Spidroins 1 and 2 and on the silk fibers themselves. The natural fibers gave very good 1-D spectra but the intensities clearly showed that to get higher dimensional data we would need to label the silk with C-13 and N-15. We have accomplished that by feeding the spiders labeled amino acids in their water. The actual incorporation, based on C-13 NMR is nearly 100% for Gly and over 95% for alanine. We have contacted the stable isotope facility at Los Alamos for collaboration in providing other labeled amino acids for use in structure determination. We are also contemplating synthesizing peptides with various positions labeled to further study them at higher dimensional NMR. The goals of this are to construct the protein structure in the fiber using distance constraints from the NMR and our molecular modeling.

A number of molecular modeling approaches have been used to study the two proteins. The data are intriguing but without any solid data they are just pretty pictures. However, the two most obvious models, a ß-spiral and a flat ß-turn sheet will generate different distances in the NMR and can clearly be differentiated. Thus, a first approximation model can be tested and then further refined as the data is generated.

In addition the above studies we have developed a number of new methods which have enabled us to carry out them out. These included: 1) a new mini-plasmid preparation method which was developed into a kit and will be sold by Baker Chemical Co.; 2) an RNA isolation method; 3) a method to remove the primers and cover oil from PCR samples; 4) an mRNA isolation method and all of the above three kits are now being sold by Amresco; 5) a phage isolation method which is substantially faster than previous methods and 5) a method for two dimensional analysis of mRNA which works like a Northern blot but is more specific.

C. (1992) Lewis, R.V. Spider Silk: The Mystery Starting to Unravel Accts. Chem. Res. 25: 392-397.
(1992) Hinman, M.B. and Lewis, R.V. Isolation of a Clone Encoding a Second Dragline Silk Fibroin, Nephila Clavipes Dragline Silk is a Two Protein Fiber J.Biol. Chem 267: 19320-19324.
(1993) Kadokami, Y. and Lewis, R.V. A Proposal for a New Language to Describe Experiments in Molecular Biology (in press, International Journal of Genome Research)
(1994) Hinman, M., Dong, Z., Xu, M. and Lewis, R.V. Spider Silk Proteins (in press, J. Materials Research)
(1994) Stauffer, S.L., Coguill, S.L. and Lewis, R.V. Comparison of Physical Properties of Spider Silks (in press, J. of Arachnology)

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E. Minor ampullate silk proteins, patent application submitted March 1994
A new method for large DNA fragment cloning, application in preparation